CLAIMS

1. A method for quantitatively detecting an antigen which comprises:

a first step of providing an Fab' antibody having a uniform isoelectric point, said antibody forming an immune complex with an antigen in an analytical sample and being modified by adding an amino acid sequence comprising a charged amino acid residue and by being labeled with a fluorescent dye;

a second step of mixing the Fab' antibody having a uniform isoelectric point with the analytical sample containing the antigen to obtain a mixture comprising the immune complex;

a third step of separating the mixture by performing electrophoresis in a carrier;

a fourth step of irradiating an excitation light which excites the fluorescent dye to the mixture separated in the third step to cause fluorescence in the immune complex; and

a fifth step of detecting the fluorescence.

2. A method according to claim 1, wherein the amino acid sequence is added adjacent to a C-terminal of an L chain of the Fab' antibody having a uniform isoelectric point.

3. A method according to claim 1, wherein the fluorescent dye is bound to a cysteine residue which is not involved in binding with an L chain and which exists in an

amino acid sequence adjacent to a C-terminal of a CH1 region of the Fab' antibody having a uniform isoelectric point.

4. A method according to claim 1, wherein the electrophoresis is performed by isoelectric focusing.

5. A method according to claim 1, wherein the electrophoresis is performed by capillary electrophoresis.

6. A method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab' antibody;

a second step of site-specifically mutating in the Fd chain gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified Fd chain gene;

a third step of linking the modified Fd chain gene and an L chain gene encoding an L chain of the Fab' antibody in the expressible state to obtain a gene expressing a modified Fab' antibody;

a fourth step of modifying the gene expressing a modified Fab' antibody to express an amino acid sequence

comprising a charged amino acid residue adjacent to a C-terminal of the L chain to obtain a gene expressing a charge modified Fab' antibody;

a fifth step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a sixth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric point obtained in the fifth step.

7. A method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab' antibody;

a second step of site-specifically mutating in the

Fd chain gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified Fd chain gene;

a third step of providing an L chain gene encoding an L chain of the Fab' antibody;

a fourth step of modifying the L chain gene to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain to obtain a charge modified L chain gene;

a fifth step of linking the modified Fd chain gene and the charge modified L chain gene in the expressible state to obtain a gene expressing a charge modified Fab' antibody;

a sixth step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a seventh step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an

L chain in the Fab' antibody having a uniform isoelectric point obtained in the sixth step.

8. A method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab' antibody, and an L chain gene encoding the L chain of the Fab' antibody;

a second step of linking the Fd chain gene and the L chain gene in the expressible state to obtain a gene expressing an Fab' antibody;

a third step of modifying the gene expressing an Fab' antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain, and site-specifically mutating in the gene expressing an Fab' antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a gene expressing a charge modified Fab' antibody;

a fourth step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab'

antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a fifth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric point obtained in the fourth step.

9. A method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing a CH1 gene encoding a CH1 region and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in a first Fab' antibody, and a CL gene encoding a CL region of the first Fab' antibody;

a second step of site-specifically mutating in the CH1 gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified CH1 gene;

a third step of cutting the modified CH1 gene with

a restriction enzyme to obtain a gene fragment encoding the CH1 region;

a fourth step of providing a VH gene encoding a VH region of a second Fab' antibody and a VL gene encoding a VL region of the second Fab' antibody;

a fifth step of linking the gene fragment, the CL gene, the VH gene and the VL gene in the expressible state to obtain a gene expressing a modified Fab' antibody;

a sixth step of modifying the gene expressing a modified Fab' antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the CL region to obtain a gene expressing a charge modified Fab' antibody;

a seventh step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a eighth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric

point obtained in the seventh step.

10. A method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing a CH1 gene encoding a CH1 region and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in a first Fab' antibody, and a CL gene encoding a CL region of the first Fab' antibody;

a second step of site-specifically mutating in the CH1 gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified CH1 gene;

a third step of cutting the modified CH1 gene with a restriction enzyme to obtain a gene fragment encoding the CH1 region;

a fourth step of modifying the CL gene to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the CL region to obtain a charge modified CL gene;

a fifth step of providing a VH gene encoding a VH region of a second Fab' antibody and a VL gene encoding a VL region of the second Fab' antibody;

a sixth step of linking the gene fragment, the charge

modified CL gene, the VH gene and the VL gene in the expressible state to obtain a gene expressing a charge modified Fab' antibody;

a seventh step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a eighth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric point obtained in the seventh step.